AN AGRONOMIC AND BIOCHEMICAL COMPARISON OF FOUR STRAINS OF CORN WHICH DIFFER WIDELY IN TOTAL GRAIN NITROGEN

BY

STANLEY ARTHUR WATSON

A.B., Pomona College, 1939
A.M., University of Illinois, 1941

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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R. UNIV. BIBLIOTHEEK LEIDEN

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Introduction

Current interest in raising the protein content of hybrid corn by breeding methods has emphasized the need for information regarding the relative field performance and biochemical differences among high, medium, and low protein strains of corn. Comparisons were made in this work among four strains which averaged 6, 10, 15, and 20 per cent crude protein in the grain.

Hopkins, East and Jones, and others showed that high and low-protein lines could be obtained from strains of average protein content by ear-to-row selection or by application of Mendelian concepts of inheritance. Hopkins' ear-to-row selection experiment culminated in two Illinois strains which now average 6 per cent and 20 per cent crude protein. Preliminary biochemical comparisons between the Illinois Low- and High-Protein strains led Hoener and De Turk to conclude that the difference between the strains was in part due to difference in ability to absorb nitrogen from the nutrient medium. Their data also showed these strains to be different in utilization of nitrogen. Clues as to the identity of a hypothetical factor in the grain which controls protein synthesis have been sought in the present work.

Materials And Methods

The Illinois High- and Illinois Low-Protein strains of corn and two Funk yellow hybrids, (11x35) Ill.H.P. and (11x35)3, were grown in 1947 in a replicated field experiment. Each strain was grown at a population of 8,000 plants an acre in five different fertility plots which included high nitrogen-low phosphorus, high N-high P, low N-high P, and intermediate

levels. Samples of sib-pollinated grain at milk, dent, and mature stages were collected at 2, 5, and 8 weeks after pollination respectively. The samples were frozen in dry ice and lyophile dried. Data are presented which show that the 8-weeks samples were mature. The acre yield of grain from each of twenty duplicate plots was obtained at 12 weeks after pollination. Chemical analyses include total nitrogen, total phosphorus, oil, nitrogen fractions, and phosphorus fractions. Nitrogen fractions include the following forms: tungstic acid -soluble (non-protein nitrogen), 5% trichloracetic acid-soluble (peptide nitrogen), salt-soluble nitrogen insoluble in 5% tricholracetic acid (globulin nitrogen), alcohol-soluble (zein nitrogen), alkali-soluble (glutelin nitrogen), and insoluble or residual nitrogen. All nitrogen precipitable with tungstic acid was termed "true protein." Phosphorus fractions included the following forms: absolute ethanol-soluble (phospholipid phosphorus), cold trichloracetic acid-soluble (phytin phosphorus), hot trichloracetic acid-soluble (nucleoprotein phosphorus), and residual phosphorus.

Results And Conclusions

The Agronomic Data. Comparative data of agronomic interest on the four strains include total crude protein, oil content, acre yield of grain, and acre yield of protein in the grain. Statistical analysis of these data showed that strains and treatments were very highly significant but that their interaction was not significant. All strains showed significant responses to nitrogen and phosphorus fertilizers. The highest protein values and protein yields were obtained on the plots of highest fertility.

A summary of the percent crude protein and pounds of protein yielded per acre is given in table I. Mean acre yields for the four strains from the high N-high P plots as bushels per acre of grain at 15.5 per cent moisture were as follows: Illinois High-Protein, 25.0; (11x35) Ill.H.P., 39.7; (11x35) 3, 52.5; Illinois Low-Protein, 34.4 Some data were also obtained on the commercial four-way cross, G-94, which was used as a

border variety within fertilizer treatments. G-94 gave results similar to those of the (11x35)3 strain, except that the grain of G-94 was about 0.5 to 1.0 per cent lower in protein. Grain yields as a whole were low because of a thin rate of planting, a late planting date, and a dry season.

TABLE I

PER CENT CRUDE PROTEIN AND ACRE YIELD OF PROTEIN IN THE GRAIN OF FOUR STRAINS OF CORN AS AFFECTED BY PHOSPHORUS AND NITROGEN APPLICATIONS

Strains	Fertilizer Treatments[1]					
	N_0P_{66}[2]		$N_{300}P_{66}$		$N_{300}P_0$	
	Crude protein %	Protein yield lbs/A	Crude protein %	Protein yield lbs/A	Crude protein %	Protein yield lbs/A
Ill. High Protein	17.8	170	20.0	279	20.0	238
(11x35) Ill. H. P.	14.3	256	16.1	388	14.5	234
(11x35)3	9.9	229	11.3	297	10.2	222
Ill. Low-Protein	5.7	88	6.5	110	5.9	80

STATISTICAL DATA[3]

Protein Percentages	Protein yield
Standard deviation = ± 0.4	Standard deviation = ± 24 lbs.
D.R.F.S.[4] = 1.0	D.R.F.S. = 67 lbs.
Coefficient of variation=4.0%	Coefficient of variation=14.6%

1) Data are means from duplication plots.
2) Subscripts refer to pounds of element applied per acre.
3) Based upon 49 degrees of freedom.
4) D.R.F.S. = difference required for significance at the 5% level.

The protein yield data were considered of greatest im-

portance in determining the potential commercial value of high-protein hybrids. Two conclusions in this regard stand out clearly: (1) a high-protein strain must have high yielding ability, and (2) the true protein-yield potential of high-protein hybrids cannot be expressed unless soil nitrogen and phosphorus are optimum. Strain (11x35) Ill.H.P. yielded only slightly more protein per acre than strain (11x35)3 when nitrogen and phosphorus were inadequate, but when these two elements approached optimum levels the high-protein hybrid produced a much greater yield of protein. These data emphasize the necessity for adequate soil phosphorus in protein production.

The percent of oil in the mature grain tended to be higher in strains having high grain protein content.

The Biochemical Data: In order to compare the overall biochemistry of the four strains, sampels were collected and analyzed at the three main stages of grain development. The grain components were expressed, (1) as per cent of dry weight, (2) as weight per ten plants (ten ears), and (3) in the mature grain samples only, as weight per 100 kernels.

Per cent total nitrogen declined with advancing maturity and reflected at all stages the final nitrogen concentration. Nitrogen fractions were estimated in grain samples at each stage of maturity from one high N-high P plot. Expressed as per cent of the whole grain or as per cent of the total grain nitrogen, zein and insoluble nitrogen increased with advancing maturity; non-protein, globulin, and glutelin nitrogen decreased; but peptide nitrogen was relatively unchanged. Concentration of practically all fractions tended to be lower in mature grain from a plot deficient in nitrogen. When mature grain of the four strains was compared, concentration of zein and insoluble nitrogen were in direct proportion to total nitrogen, whereas globulin decreased with increasing grain nitrogen. Expressed as per cent of the total nitrogen, zein and glutelin nitrogen were present respectively in the grain of the four strains as follows: Ill. High-Protein, 55.0,

12.1; (11x35) Ill.H.P., 50.2, 15.9; (11.35)3, 42.9, 21.2; Ill. Low-Protein, 25.0, 26.9.

Rates of accumulation of constitutnts on a plant-weight basis showed striking differences among the strains. The Illinois High-Protein strain was deficient in rate and extent of accumulation of total dry substance. The Ilinois Low-Protein strain was deficient in protein accumuation. The latter strain had accumulated almost its entire weight of protein by the second week after pollination, whereas the other three strains had accumulated the greatest amount of protein between the second and fifth weeks. The rate of accumulation of zein was positively correlated with the total true protein in the mature grain of the four strains. The inheritance of high protein character in corn appears to be largely the inheritance of greater zein synthesis. However, since high-protein strains are lower in yield of dry substance, high protein content might also be the result of inefficient starch synthesis. Starch was estimated by difference [total dry substance — (crude protein + oil)] and was found to be inversely related to protein content of the grain. Starch in the grain of the four strains as estimated by difference was: Ill. High-Protein, 13.4; (11x35) Ill.H.P., 20.7; (11x35)3, 23.3; Ill. Low-Protein, 24.2 grams per 100 kernels.

Alkali-insoluble nitrogen was estimated in all strains at the three stages of maturity. The concentration of insoluble nitrogen was positively correlated with total true protein in the mature grain only. To investigate the possibility that this fraction contained a substance which might regulate protein synthesis in the grain, a large sample of the alkali-insoluble fraction was prepared and subfractionated. There was no evidence for the presence of a regulator substance in any of the subfractions. It was concluded that the nitrogen in this fraction was in part derived from the hull but was mostly the resultant of incomplete removal of zein and/or glutelin from the germ or endosperm during fractionation operations. The large variations in amounts of alkali-insoluble nitrogen obtained by previous investigators has been partially

explained.

A positive correlation was found between the total phosphorous and total true protein in the mature grain of all strains whether grown on soil of high or of low phosphorus levels supplied with adequate nitrogen. Phosphorus compounds were fractionated in samples of mature grain from the high N-high P plot. The entire phosphorus metabolism of the high-protein strains appeared to be more intense than that of the low-protein strains. Phytin, nucleoprotein, and residual phosphorus were all correlated with the total nitrogen. Nucleoprotein phosphorus, which made up about 9 per cent of the total phosphorus of the mature grain, was of special interest because of its close connection with protein synthesis in various animal and plant cells. Nucleoprotein phosphorus content of milk stage and mature grain of all strains was positively correlated with the total true protein content. The relationship was interpreted as support for the hypothesis that nucleoprotein may regulate protein synthesis in the grain. More work needs to be done, however, to strengthen and extend this line of evidence.

Mature grain of the four strains from the high N-high P plot were dissected into three parts — hull plus tip-cap, germ, and endosperm — and were analyzed for total nitrogen and total phosphorus. The endosperm nitrogen of each strain constituted the greatest proportion of the total nitrogen in the whole grain and varied directly with the latter. The germ of each strain was found to contain from 60 to 80 per cent of the phosphorus of the entire grain. Germ phosphorus was directly proportional to the total grain phosphorus.

Summary

Four strains of corn which averaged 6, 10, 15, and 20 per cent crude protein in the grain were grown in field plots fertilized with varying amounts of phosphorus and nitrogen. The high-protein hybrid on the plot of highest fertility contained 16.1 per cent crude protein and produced more protein

per acre than any of the other strains. This strain, however, was outstanding only on the plots which had received the greatest amounts of phosphorus and nitrogen fertilizers. The importance of phosphorus in protein production was emphasized.

The inheritance of high protein character in the grain is apparently an inheritance of greater capacity for zein synthesis and a possible inhibition of the ability to accumulate starch. The Illinois Low-Protein strain makes very little zein and accumulates the bulk of its final protein in the first two weeks after pollination.

The correlation found between alkali-insoluble nitrogen and total true protein appeared to be due to the incomplete removal of zein and/or glutelin by the fractionation procedure employed.

Phosphorus metabolism of the high-protein strains appeared to be unusually intense since all phosphorus fractions were higher in the high protein strains. Sixty to eighty per cent of the total phosphorus in the grain of all strains was found in the germ. Nucleoprotein phosphorus in the whole grain was directly correlated with total true protein at the two stages of maturity studied — milk and mature. This observation suggested that nucleoprotein may be a regulator of protein synthesis in corn grain.

VITA

Stanley Arthur Watson was born in Los Angeles, California, on August 30, 1915. The entire elementary and secondary school education was received in the schools of Pomona, California. In 1933 he entered Pomona College, Claremont, California, but withdrew after two years to assist his father in the establishment of a retail feed business. In 1937 the author returned to Pomona College to complete the work for an A. B. in chemistry, which degree was received in 1939. In the fall of 1939 he entered graduate study in plant physiology in the Department of Botany, University of Illinois, where he was employed half-time as a teaching assistant. An A.M. degree in botany was received in 1941.

During the years, 1942 to 1946 the author was employed as chemist in the Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Illinois, where he conducted research on starch structure. In July 1946 he returned to the University of Illinois as a research assistant in the Division of Soil Fertility, Department of Agronomy, to complete the requirements for the Ph.D. degree. Following completion of graduate study he took employment in the

Chemical Division of Corn Products Refining Co., Argo, Illinois, to conduct research on the steeping of corn.

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